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Environmental Consequences of Nanotechnologies

Quantifying Nanoparticle Release from Nanotechnology

Scientific Operating Procedure Series: SOP-C-3

David P. Martin, Aimee R. Poda, and Anthony J. Bednar

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Abstract

Material that is released from nanotechnology during testing can be a complex mixture of nanoparticles, dissolved species, matrix-embedded particles, and particles with sizes outside of the nano range. Thorough characterization of the released material is crucial to predicting its toxicity. This report provides a general framework for determining not only whether material is released from a nanotechnology during testing, but also whether the released material can still be considered a nanomaterial. The framework is written in broad terms so that it can theoretically be applied to any material, but references are provided for documents specific to the more common nanomaterials and detection methods. It is the authors intent that this document be used in conjunction with previously established methods and procedures to help guide the user through the characterization process following release testing of a nanotechnology.

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Preface

This procedure was prepared for the U.S. Army Corps of Engineers, Headquarters (USACE HQ) under the U.S. Army Engineer Research and Development Center (ERDC) Environmental Quality and Technology (EQT) Research Program titled “Environmental Consequences of Nanotechnologies.” Procedures discussed in this report link to the ERDC NanoGRID (Guidance for Risk Informed Deployment) framework for testing the exposure and hazard of nanotechnology.

This work was coordinated by the Environmental Chemistry Branch (EPC) of the Environmental Processes and Engineering Division at the ERDC - Environmental Laboratory (ERDC-EL). David Morrow was the Branch Chief, Warren Lorenz was the Division Chief, and Dr. Elizabeth Ferguson was the Technical Director for Military Environmental Engineering and Science. The Deputy Director of ERDC-EL was Dr. Jack Davis and the Director was Dr. Elizabeth Fleming

COL Bryan S. Green was Commander of ERDC and Dr. Jeffery P. Holland was the Director.

Definitions

Agglomerate	in nanotechnology, an assembly of particles held together by relatively weak forces (for example, Van der Waals or capillary) that may break apart into smaller particles upon processing
Nanomaterial	material with at least one dimension between 1 and 100 nm
Nanotechnology	for the purposes of this Scientific Operating Procedure (SOP), any product or technology that contains a nanomaterial
Parent nanomaterial	the nanomaterial of interest existing in its pure form prior to inclusion into a product or technology
Particulate	components of a liquid suspension that are greater than 1-nm in size

Acronyms

AES	atomic emission spectroscopy
AFM	atomic force microscopy
ASTM	American Society for Testing and Materials
CE	capillary electrophoresis
CPC	condensation particle counter
DLS	dynamic light scattering (also known as PCS)
EHS	environmental health and safety
FFF	field flow fractionation
ICP	inductively coupled plasma
MS	mass spectrometry
ISO	International Organization for Standardization
MALDI	matrix-assisted laser desorption/ionization
NTA	nanoparticle tracking analysis
PCS	photon correlation spectroscopy (also known as DLS)
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
SEC	size-exclusion chromatography
SEM	scanning electron microscopy
SLS	static light scattering
SP	single-particle
TEM	transmission electron microscopy
TOF	time of flight
U.S. EPA	United States Environmental Protection Agency
UV-Vis	ultraviolet-visible

1 Introduction

The SOP described herein for assessing the properties of nanotechnologies was developed under Task 2: Optimized Scientific Methods of the ERDC/EL Environmental Consequences of Nanotechnologies research program. The primary goal of this Task was to develop robust SOPs for investigating the environmental health and safety (EHS)-related properties of nanotechnologies including nanomaterials and products incorporating nanomaterials.

Specifically, this SOP provides guidance for both determining whether nanomaterial is released from a nanotechnology during testing as well as how to characterize any released material. This SOP combines best laboratory practices available from the literature and research experiences of ERDC scientists.

2 Background

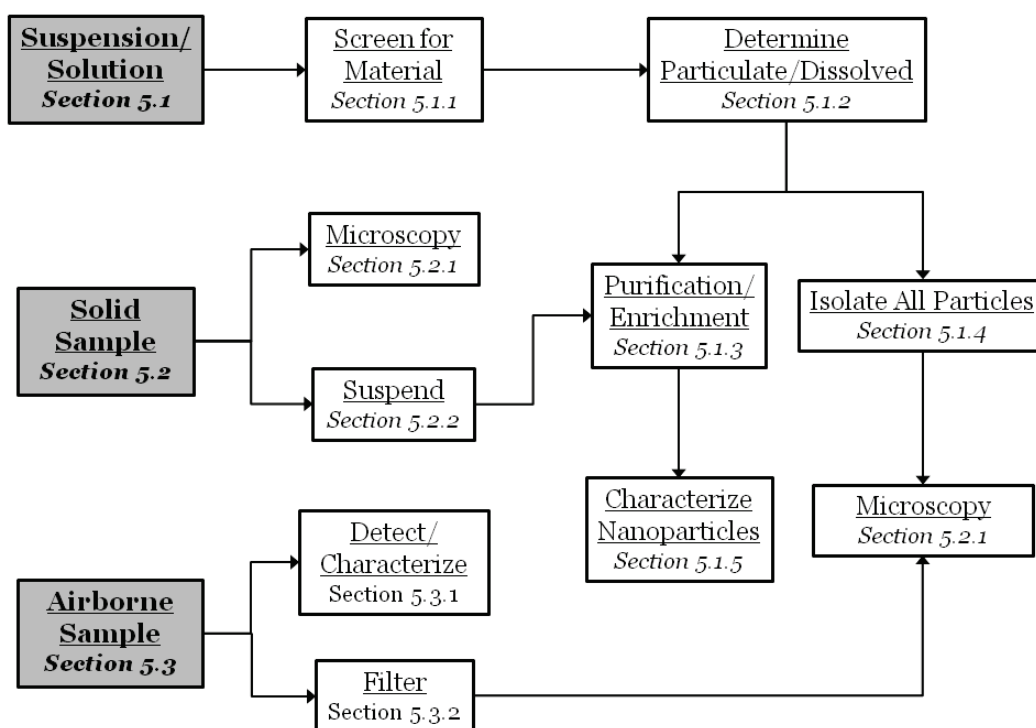
The health and environmental hazard of engineered nanomaterials can depend on a variety of factors including chemical makeup, particle size, and surface characteristics (Lin et al., 2010). Any assessment of the risks associated with a nanotechnology must take all of these factors into account, which makes rigorous characterization of the nanomaterials absolutely critical. To accommodate this need for information, there has been a steady increase in the number of reports describing novel or improved characterization methods for nanoparticles (Hassellöv et al., 2008). This has been followed by the improvement in both models that predict the ultimate fate of nanoparticles in the environment as well as our understanding of their mechanisms of toxicity.

To date, however, a vast majority of the EHS testing of nanotechnologies has been performed on the parent nanomaterial as it exists prior to incorporation into the nanotechnology (Brame et al., 2015; Froggett et al., 2014). It is important to note that the material released from the nanotechnology during use is not necessarily identical to the parent nanomaterial that is incorporated into the product; the same physical stresses responsible for the release of the material may also alter its physical and/or chemical structure (Nowack et al., 2012).

3 Scope

This SOP provides guidance for characterizing material post-release from a nanotechnology, whether in the form of a suspension or solution, solid, or airborne particles (Figure 1). This SOP is primarily focused on the overall framework and the procedure for taking a sample from testing through analysis. References for standard methods applicable to the more common nanomaterials and detection methods are given when possible, but it is ultimately up to the user to select the appropriate techniques and methods for the particular material of interest. It is recommended that users select the appropriate sections of the SOP to follow given the nature of their specific material.

Figure 1. Conceptual flow chart of the procedures discussed in this SOP.



4 Materials and Apparatus

The materials and apparatus needed will depend on the specific system under investigation and can be found in the references provided for each individual analysis technique.

5 Procedure

This procedural framework is divided into three sections, depending on the nature of the material that is generated by the release testing:

1. For liquid samples (most likely aqueous suspensions/solutions), the presence of the material of interest is confirmed by a bulk method (Section 5.1.1) prior to performing more laborious characterization methods. Once the presence of the material in bulk is confirmed, the fraction of that material that is on the nanoscale is characterized. We suggest that this be performed by using a suspension-based method (following purification, Sections 5.1.3 and 5.1.5) as well as by isolating all non-dissolved species in the solid phase and characterizing them by microscopy (Sections 5.1.4 and 5.2.1).
2. For solid samples, where the presence of released material is more obvious, the material is first characterized by microscopy (Section 5.2.1) and then suspended (Section 5.2.2) and analyzed as described for liquid samples in Step (1).
3. For airborne samples, the presence of released material is first verified and characterized by an appropriate method (Section 5.3.1). If possible, the released particles are then filtered or otherwise isolated in a solid phase that can be analyzed by microscopy (Section 5.2.1).

It should be noted that the steps outlined in this procedure do not necessarily need to be performed in order; the reader is directed to Figure 1 for more guidance.

5.1 Procedures for Suspension/Solutions

5.1.1 Detection of the material of interest in bulk

To avoid unnecessary testing, the recommended first step in analyzing a liquid sample is a relatively fast and inexpensive test to confirm the presence of the material of interest. This can be accomplished using a variety of techniques, the most common of which are listed in Table 1. One of the most frequently used methods to confirm the presence of nanoparticles is to determine the turbidity of the sample, which measures the amount of light scattered by particles in suspension (ASTM, 2011a; International Organization of Standardization (ISO), 1999). The primary

drawbacks to using turbidity are the relatively high limit of detection (low parts per million (ppm) to high parts per billion (ppb) concentrations) and the fact that it does not supply information about the composition or size of the particles. False positives could potentially be caused by other particles (even outside of the nano range) that are released from the nanotechnology. Static and dynamic light scattering (SLS and DLS, respectively) are related techniques that give additional information on the particle size, and can thus verify the presence of nanoparticles, but still do not verify the chemical makeup of the material (ASTM, 2015; ISO, 2009; ISO, 2008). Since the chemical makeup of the nanomaterial is generally known, a more definitive approach is to look for its chemical signatures. If the nanoparticles of interest contain a metal or metalloid, inductively coupled plasma mass spectrometry (ICP-MS) or atomic emission spectroscopy (ICP-AES) should be performed on an acid-digested sample to test for the presence of those elements known to make up the nanoparticles (ISO, 2011c; United States Environmental Protection Agency (U.S. EPA) 2000; U.S. EPA, 2014). With detection limits in the low ppb range, these techniques are much more sensitive than turbidity and have the added benefit of positively identifying the elements of interest in the test sample.

Table 1. Some of the more frequently used methods for detection of common nanomaterials in liquid samples.

Analysis Method	Approximate Limit of Detection	Materials Detected
Turbidity, DLS, or SLS	ppm	All light-scattering particles
Atomic Spectroscopy	ppt-ppb	Metals and metalloids
Optical Absorption Spectroscopy	ppb-ppm	Ag, Au, carbon nanotubes, graphene, graphite, Fullerenes, quantum dots
Emission Spectroscopy	ppb	Fullerenes, quantum dots
Mass Spectrometry	ppb-ppm	Vaporizable materials

In addition, there are also techniques that are more specific to certain materials. Some metallic nanoparticles, such as silver and gold, exhibit surface plasmon resonance that gives rise to characteristic absorbance bands in the visible region; this can be quantified using optical absorption spectroscopy to determine not only concentration of the particles, but also their size (ISO, 2015c; Creighton and Eadon, 1991; Kelly et al., 2003). Aromatic organic species such as carbon nanotubes, graphene, or

fullerene-like materials can potentially be detected by their absorbance in the UV region (Chen et al., 2008). Less common techniques include emission spectroscopy for fluorescent or phosphorescent materials (An et al., 2002) and Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry (Khitrov and Strouse, 2003). The selection of characterization methods will be ultimately be dependent on the target material, lab capacity, and user preference.

5.1.2 Quantification of dissolved and particulate fractions

Before proceeding to more in-depth characterization of particle size for the material of interest, the ratio of particulate to dissolved species should be determined. Not only is this information useful to understanding the mechanism of release from the nanotechnology, but it is also crucial to the analysis of any toxicity data obtained for the sample. The most common and broadly applicable method for separation of particulate and dissolved species is ultracentrifugation, which is discussed further in Section 5.1.4 (Kennedy et al., 2012; Kennedy et al., 2010). As an alternative, for some ions, such as Ag^+ and Au^+ , there are ion-specific electrodes that can directly measure the concentration of dissolved ions in solution (Gupta et al., 2011). However, these measurements are susceptible to interferences, and the range of ions that can be sensed by this method is relatively small.

5.1.3 Purification/enrichment of nanoparticles in suspension

In some cases, the concentration of nanoparticles in samples from release testing may not be high enough to be detected by the characterization technique to be used (discussed in Section 5.1.5) and thus must be concentrated prior to analysis. If the nanoparticles have sufficient stability, simply evaporating the solvent is a viable option to increase the concentration. The user should take into account that this will also concentrate any other species present in the solution, which may have implications on the stability of the suspension. Ultrafiltration, in which the solvent and dissolved species pass through a membrane with a very low size cutoff, allows for the concentration of particles without increasing the concentration of dissolved species. Drawbacks to this technique include the loss of smaller particles through the membrane and the potential for nanoparticles adhering to the membrane due to the large pressures applied across it.

Diafiltration and tangential flow ultrafiltration are more gentle techniques for enrichment and purification based on the ultrafiltration concept (Dalwadi et al., 2005; Sweeney et al., 2006). In these techniques, the sample suspension is pumped perpendicular to a membrane so that only a fraction of the sample permeates through it (Houp, 2009). Because the system pressure is not directed entirely across the membrane in these techniques, the loss of smaller nanoparticles and adhesion to the membrane may be diminished, leading to a higher yield. Utilizing several membranes in a step-wise fashion allow the techniques to be used to remove large particles or agglomerates that may interfere with subsequent characterization techniques, in addition to removing any undesirable dissolved species.

Ultracentrifugation can also be used to enrich a nanoparticle sample and remove dissolved species (Miller et al., 2014). Concentration of the sample can be accomplished by centrifuging at conditions less than those necessary to pellet the nanoparticles followed by decanting of the upper portion of the sample, from which particles have been removed. Subsequent steps of centrifugation, dilution with a desired solute, and further centrifugation can be used to manipulate the concentration of dissolved species in the sample.

5.1.4 Isolation of particulate species from suspension

In addition to characterizing the nanoparticles in suspension, it can be informative to characterize them by microscopy (discussed in Section 5.2.1). The requirements for sample preparation will vary depending on the method to be used, but in all cases, the nanoparticles must be isolated from the suspension prior to analysis. A straightforward way to accomplish this is to filter the sample using a membrane with a very small size cutoff and image what is captured on the surface.

Since smaller particles will inevitably pass through the membrane, filtration is sufficient only if qualitative data is desired; for more quantitative analysis, a technique that isolates all of the particles from suspension is necessary. Ultracentrifugation can accomplish this, although there are still limitations. The time necessary to force particles to settle out of suspension is dependent on the mass of the particles, so both material density and particle size have a significant impact on the effectiveness of the technique. Small particles (<10-nm) and those with low density are difficult to separate by

this technique; recent advancements in the instrumentation to allow for larger centrifugal forces have diminished these issues.

5.1.5 Size determination of suspended nanoparticles

There are a variety of techniques available to characterize suspended nanoparticles (Hassellöv et al., 2008), and it is recommended that a sample be analyzed by at least two distinct methods (Table 2). Several techniques are used to analyze the Brownian motion of the particles (e.g. DLS and NTA); since these techniques require only that the particles scatter light, they are applicable to a vast majority of materials. Another set of methods accomplish size characterization by coupling a technique that separates the particles based on size (e.g. FFF or size-exclusion chromatography) to a detection method for the material of interest (described in Section 5.1.1). Other techniques are more specific to the material, such as absorption spectroscopy for silver and gold (particles that display surface plasmon resonance) or X-ray diffraction and raman spectroscopy for highly crystalline materials.

Table 2. Methods for size characterization of nanomaterials.

Analysis Method	Method References	Applicable Materials
Light scattering (DLS/PCS, SLS)	(ASTM, 2015; ISO, 2009; ISO, 2008)	All light-scattering particles
FFF ^a	(Bednar et al., 2015; Bednar et al., 2013; Poda et al., 2011)	Detection-dependent
SP-ICP-MS	(ISO, 2015d; Bednar et al., 2015; Laborda et al., 2014; Mitrano et al., 2012)	Metals and metalloids
Optical Absorption Spectroscopy	(ISO, 2011b; Amendola and Meneghetti, 2009; Haiss et al., 2007)	Ag, Au
Fluorescence correlation spectroscopy	(Domingos et al., 2009; Zhang et al., 2005)	Fluorescent materials
Raman Spectroscopy	(Gouadec and Colomban, 2007; Stiles et al., 2008)	Crystalline materials
X-ray Diffraction	(ISO, 2015a)	Crystalline materials
Liquid Chromatography (SEC, CE) ^a	(Fedotov et al., 2011)	Detection-dependent
Nanoparticle Tracking Analysis	(ASTM, 2012b)	All light-scattering particles

5.2 Procedures for Solid Samples

Some release tests, such as abrasion, may generate a solid sample that inherently indicates that material was released from the nanotechnology. The material is first characterized in the solid state (ISO, 2013), then suspended and characterized as discussed in Section 5.1 to determine its behavior in an aqueous system.

5.2.1 Size Characterization

The first step to characterizing a solid sample from a release test is to observe it using microscopy and determine the overall morphology of the sample. While optical microscopy can be useful in determining if a majority of the released material is larger than the nano-scale, it does not have sufficient resolution necessary to characterize nano-sized particles. Electron microscopy, including SEM (ISO, 2011a) and transmission electron microscopy (TEM) (ISO, 2012a), can provide both high resolution images as well as some elemental information through energy dispersive X-ray analysis (ASTM, 2012a). Another option for visualizing the sample is through atomic force microscopy (AFM) (ASTM, 2011b), which can provide resolution on the same level as TEM (<1 nm), but does not have the same potential for elemental analysis.

The main drawback to microscopy as a method for size characterization is that it analyzes only a small fraction of the material that may or may not be representative of the entire sample, although automated software has made taking a large number of measurements more efficient, which can allow for robust statistical analysis. There are very few techniques for the size characterization of a bulk solid sample. The most common method, gas sorption, measures the specific surface area of the particles, which can then be correlated to a particle size (ISO, 2010; Hassellöv et al., 2008) .

5.2.2 Suspension

Since analysis methods for a solid sample are limited and the environmental impact of released material will certainly depend on its behavior in water, it is recommended that the sample be suspended (Coleman et al., 2015) and characterized as described in Section 5.1.

5.3 Procedures for Airborne Samples

Inhalation of airborne nanoparticles represents one of the most significant hazards arising from nanotechnology (Bello et al., 2009). The characterization of airborne particles, however, is very challenging. A handful of technologies exist that can detect airborne particles, and some can even provide size characterization data. The recommended procedure for airborne samples involves first detecting the particles in the air (ISO, 2012b), and, if possible, filtering the particles for complementary characterization.

5.3.1 Particle Detection and Characterization

The most common technology for detecting airborne nanoparticles is condensation particle counting (ISO, 2015b); a process in which a low vapor pressure material is condensed onto the particles to bring them up to a size that can be detected by optical methods. Elaborations on this method, including differential mobility particle sizing and scanning mobility particle sizing, can provide size information as well as detection by ionizing the particles and interacting them with an electric field prior to introduction to the condensation particle counter (CPC) (Kaminski et al., 2013). Fast mobility particle sizing also ionizes the particles and detects the particles *in situ* with electrometers rather than a CPC, which allows for much faster sampling times, but with lower sensitivity. Electrical low pressure impactors also ionize the particles, but are separated by aerodynamic diameter rather than electrical mobility (Arffman et al., 2014).

5.3.2 Filtration

In addition to *in situ* detection, the airborne particles should be isolated and characterized in the solid state as discussed in Section 5.2. Filtration is by far the most common method for isolating airborne nanoparticles (Kim et al., 2007). Alternatives include impactors and electrostatic precipitation.

6 Reporting

6.1 Analysis of Results

The analysis and reporting of results are highly method-dependent and are discussed in the references provided. Additionally, details concerning the specific protocols used to simulate release scenarios should be included with the chemical and physical analysis results, as that will aid in the determination of ultimate health or environmental impact.

6.2 Key Results Provided

The present protocol provides a framework for characterizing material that is released from a nanotechnology during testing and can be applied to solid samples, liquid suspensions, or airborne particles. Users are referred to suggested analytical methods where possible.

6.3 QA/QC Considerations

The behavior of nanoparticles can often be affected by a wide variety of experimental conditions. These considerations can be found in the reference materials.

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14. ABSTRACT Material that is released from nanotechnology during testing can be a complex mixture of nanoparticles, dissolved species, matrix-embedded particles, and particles with sizes outside of the nano range. Thorough characterization of the released material is crucial to predicting its toxicity. This report provides a general framework for determining not only whether material is released from a nanotechnology during testing, but also whether the released material can still be considered a nanomaterial. The framework is written in broad terms so that it can theoretically be applied to any material, but references are provided for documents specific to the more common nanomaterials and detection methods.						
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